Cerebrospinal fluid parameters of B cell-related activity in patients with active disease during natalizumab therapy

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Abstract: Recently, the disappearance of oligoclonal bands (OCBs) from the cerebrospinal fluid (CSF) of a few natalizumab-treated patients with multiple sclerosis (MS) has been reported. This is interesting since CSF-restricted OCB are believed to persist in MS. We pooled CSF data from 14 MS centers to obtain an adequate sample size for investigating the suspected changes in central nervous system (CNS)-restricted humoral immune activities in the context of natalizumab therapy. In a retrospective chart analysis, CSF parameters of blood-CSF barrier integrity and intrathecal IgG production from 73 natalizumab-treated MS patients requiring a diagnostic puncture for exclusion of progressive multifocal leukoencephalopathy were compared with CSF data obtained earlier in the course of disease before natalizumab therapy. At the time of repeat lumbar puncture, local IgG production (according to Reibergram) was significantly reduced ($p < 0.0001$) and OCB had disappeared in 16% of the patients. We therefore conclude that natalizumab therapy interferes with intrathecal antibody production at least in a significant number of patients.

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SHORT REPORT for resubmission to **Multiple Sclerosis Journal**

Cerebrospinal fluid parameters of B cell-related activity in patients with active disease during natalizumab therapy

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ABSTRACT

Recently, disappearance of oligoclonal bands (OCB) from the CSF of a few natalizumab-treated patients with MS has been reported. This is interesting since CSF-restricted OCB are considered to persists in MS.

Since lumbar punctures during natalizumab therapy are infrequent we pooled CSF data from fourteen MS centers to obtain a suitable patient cohort for furhter investigations about the suspected impact of natalizumab on CNS-restricted humoral immune activities.

In a retrospective chart analysis CSF parameters of blood-CSF barrier integrity and intrathecal IgG production from 73 natalizumab-treated MS patients requiring a diagnostic puncture for exclusion of progressive multifocal leukoencephalopathy were compared with CSF data obtained earlier in the course of disease before natalizumab therapy.

Introduction

Natalizumab is an effective monoclonal antibody therapy for the treatment of relapsing- remitting multiple sclerosis (RRMS) and interferes with immune cell migration into the CNS by binding to the adhesion molecule very late activation antigen-4 (VLA-4).[1] The result is a pronounced and sustained reduction of immune cell numbers in the CSF.[2]

Less knowledge exists about natalizumab interfering with intrathecal humoral immune activities. Recently, disappearance of oligoclonal bands (OCB) during natalizumab therapy in 4 out of 6 patients was reported from von Glehn et al.[3] This is very interesting and substantiates the importance of investigating intrathecal antibody production in response to natalizumab treatment because, in contrast to other CNS diseases including neuromyelitis optica[4], CSF-restricted OCB have been reported to persist in MS.[5,6]

Since CSF data from patients during natalizumab therapy is scarce, we collected CSF data from several specialized MS centers to obtain an appropriate sample size for determining significant changes in CNS-restricted humoral immune activity attributable to natalizumab therapy.

Patients and methods

Patients

CSF parameters from 73 patients (mean age 37.0, range 17-65) with RRMS were collected retrospectively at fourteen German, Swiss, and Austrian hospitals. Lumbar punctures were performed (i) at first diagnosis of MS and/or other routine diagnostic purposes before natalizumab therapy, and ii) for diagnostic purposes after an average of 30 months (SD 17.9) on natalizumab therapy, in particular, to rule out progressive multifocal leukoencephalopathy (PML). In 7 patients (10%) presence of PML was confirmed by the detection of JC virus DNA by PCR. In 16 patients natalizumab therapy was terminated or paused leading to intervals of >8 weeks between the last natalizumab infusion and lumbar puncture.

CSF parameters

CSF data were obtained from MS centers whose laboratories participate at interlaboratory testing and/or are certified by the German Society of CSF Diagnostics and Clinical Neurochemistry (Deutsche Gesellschaft für Liquordiagnostik und klinische Neurochemie, DGLN). IgG and albumin concentrations of CSF and serum samples were measured by immunonephelometry and used to calculate the CSF/serum quotients for IgG and albumin (QIgG, QAlb). Blood-CSF barrier integrity was assessed by QAlb with the upper reference limit of the age-dependent QAlb calculated according to Reiber et al.[7] Intrathecal IgG synthesis was quantified as the relative intrathecal IgG fraction (IgG_{IF}) using the CSF/serum quotient diagrams according to Reiber[6]. Since quantitative data were not available in several cases IgG_{IF} and QAlb were dichotomized into normal or pathologic for statistical analysis. Detection of OCB was performed by isoelectric focusing and presence or absence of OCB in the CSF but not in serum was evaluated qualitatively.

Statistics

McNemar's test was used to compare differences in CSF parameters from the two lumbar punctures. Person-Cloppers values were used to compute 95% confidence intervals (CI) for

the difference of proportions. A p-value less than 0.05 indicates a statistically significant difference. Computations were done using StatXact 6.0 (Cytel Software Corporation, Cambridge MA).

Results

Due to the retrospective design of our study some CSF parameters, mainly of the first lumbar puncture, were not available. For statistical analysis only patients with data available from both lumbar punctures were included. Accordingly, comparative analysis of blood-CSF barrier integrity was performed from 42 patients and of IgG_{IF} from 40 patients. Disappearance of OCB was evaluated from 73 patients. An overview of the results is given in the table.

At the first lumbar puncture an increased QAlb was determined in 26% (11/42) and local IgG production (represented by IgG_{IF}) was measured in 80% (32/40) of the patients. OCB were positive in 63 and unknown in 10. These 10 patients were OCB positive at the 2nd lumbar puncture and therefore assumed to be positive at the time of the 1st lumbar puncture. At the second lumbar puncture the frequency of blood-CSF barrier dysfunction was 17% (4/42) indicating little or no effects of natalizumab therapy on the blood-CSF barrier integrity (p=0.15). The frequency of IgG_{IF} was only 55% (18/40) at the second measurement and significantly lower compared to the 80% at the first lumbar puncture (p<0.0001; observed difference: 35%, 95% CI for the difference: 22-50%). OCB had disappeared in 16% (12/73) of the patients (p<0.003; observed difference: 16%, 95% CI for the difference: 9-27%) who were OCB positive at the first lumbar puncture. Very similar results were obtained upon excluding patients with confirmed PML and patients with an interval of more than 8 weeks between the last natalizumab infusion and lumbar puncture.

Particularly interesting were 2 cases with disappeared OCB despite an interval of 5.5 and 6 months since the last natalizumab infusions and 1 patient with confirmed PML in whom OCB had disappeared during natalizumab therapy but had reappeared following plasma exchange (PLEX) therapy (personal communication M. Linnebank).

Discussion

Our results corroborate findings from a small study in 6 patients by von Glehn at al.[3] and provide further evidence that natalizumab therapy has an effect on B cell activity and antibody production within the CNS. The pivotal question concerns the relevance of decreased intrathecal IgG synthesis and disappearance of OCB in response to natalizumab therapy. All the more so, as this effect apparently is specific to natalizumab since selective targeting of B cells by rituximab or arresting lymphocytes within lymph nodes by fingolimod were reported not to affect intrathecal IgG synthesis or OCB.[8-10] Absence of immunologic crosstalk due to natalizumab-mediated blockade of VLA-4, interference with plasma cell attachment to their CNS niches, or unknown direct effects of natalizumab on CNS B-lineage cells are all possible mechanisms though speculative.

Both studies – the one of von Glehn at al.[3] and ours - included one case of PML in whom OCB had disappeared at the time of PML diagnosis but became positive again after plasma exchange (PLEX) therapy which indicates a possible involvement of immune cell interactions in the maintenance of intrathecal IgG production. Apparently, re-availability of immune cells facilitated a re-boost of intrathecal IgG synthesis. Contrary, OCB still were disappeared in two of our patients despite an interval of about 6 months between the diagnostic lumbar puncture and the last natalizumab infusion. Natalizumab therapy possibly also effects a prolonged impact on intrathecal antibody production, similar to or maybe because of the sustained decrease in immune cell availability as reported by Stueve et al[2].

The retrospective design with laboratory results from different centers and the bias of a diagnostic lumbar puncture mainly performed to rule out PML certainly are limitations of our study. On the other hand, data were provided from specialized MS centers with certified laboratories/laboratories participating at interlaboratory testing and we achieved a suitable patient cohort for statistical analysis though lumbar punctures from patients under natalizumab therapy are infrequent.

In conclusion, our larger cohort confirms that natalizumab therapy affects B cell-derived activity within the CNS. However, a conclusion on disease- or treatment-relevance of this finding cannot be drawn at this point. Clarification of the underlying mechanisms and a prospective study including patients with a stable disease course could provide further knowledge on efficacy and mode of action of natalizumab therapy.

Table. Summarized CSF parameters from patients with available data of both lumbar punctures (before and during natalizumab therapy) and estimates on significance of observed changes

	1st puncture	2nd puncture	McNemar Test		
	n [%]	n [%]	n	p-value	% difference (95% CI for difference)
Evidence of intrathecal IgG synthesis					
Intrathecal synthesized fraction determined by Reibergram					
Normal	8 [20%]	22 [45%]	40	p<0.0001	35% (22-50%)
Pathologic	32 [80%]	18 [55%]			
OCB determined by isoelectric focussing					
Negative	0 [0%]	12 [16%]	73	p<0.003	16% (9-27%)
Positive	73 [100%]*	61 [84%]			
Blood-CSF barrier function (QAlb)					
Normal	31 [74%]	35 [83%]	42	p=0.15	9% (-24-4%)
Pathologic	11 [26%]	7 [17%]			

Abbreviations: CI, confidential interval; CSF, cerebrospinal fluid; IgG, immunoglobulin G; n, number of patients; QAlb, albumin quotient; OCB, oligoclonal bands

*of 10 patients OCB were not stated and assumed positive.

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